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Ver 10 9/16

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STIC-ILL

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VAN ANTWERP, W. P. et al.,

"IMPROVED INSULIN FOR IMPLANTABLE PUMP THERAPY"

Horm Metab Res 29 A11, 1997 (ABSTRACT)

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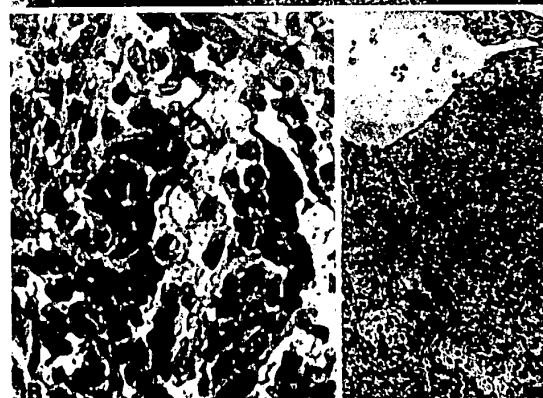
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P2

Improved Insulin for Implantable Pump Therapy

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Over the past three years, several new insulin variants have been synthesized and tested for physical stability in implantable insulin pumps. Improvements in the purification process of the insulin manufacturing has resulted in insulin that is both chemically and physically more stable than insulin from three years ago.

Because the fundamental physical reasons for insulin physical stability in the implantable pump are not fully understood, we have investigated the behavior of a variety of insulin formulations *in vitro*.

We describe several new test methodologies for evaluation of insulin physical stability and results for a number of formulations. A mathematical model of insulin physical stability in pumps has been developed and the results of the model have been used to predict the stability of insulin in pumps. The interaction of various insulin formulations with the materials of the pump has been investigated and a comparison of formulations will be discussed.

Circular dichroism, High Performance Liquid Chromatography, UV absorbance and fluorescence spectroscopy, Dynamic Light Scattering, Raman Spectroscopy and electrochemical measurements have all been used to characterize the insulin behavior in solution.

P3

On-Line Continuous i.v. Glucose Monitoring during Hemodialysis: Benefits vs. Problems

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Introduction: Diabetes mellitus is one of the major causes of end stage renal failure. Glucose toxicity, i.e. damage caused by extreme glucose excursions, may further aggravate the status of these patients. The practice of continuous i.v. glucose monitoring during hemodialysis may show the actual metabolic state of the patient and may help and correct possible hypo/hyperglycemias. **Aim:** To-evaluate the benefits vs. problems of the on-line continuous monitoring of blood glucose during hemodialysis in NIDDM using the i.v. Glucosensor Unitec Ulm. **Methods:** A catheter (0.25-0.50mm) was inserted into the tubes of the artificial kidney, located immediately after the inlet of the heparin supply pump for continuous blood extraction ($v=20-40\mu\text{l}/\text{min}$). Blood was diluted 1:5 with a heparin-NaCl solution (100U/ml) and pumped into a flow chamber containing a GOD-membrane. The membrane was attached to a Ag/Pt electrode polarized at 700mV. Glucose is oxidized in H_2O_2 and the latter releases electrons which are proportional to the glucose concentration and are measured as an electric current. A total of 8 experiments were carried out. Blood glucose was continuously monitored during hemodialysis in 6 NIDDM patients (2 woman and 4 men). **Results:** 5 to 10ml of blood were extracted in each experiment. Blood glucose fluctuated between 80 and 350mg/dl. The sensor signal correlated very well with the reference glucose values. In 2 patients, blood glucose was corrected with i.v. insulin injection during hemodialysis. The major problem was the interruption of the continuous blood extraction due to blood clotting in the tubing system of the glucosensor ($n=3$). However, this problem was avoided by perfusing 2000U heparin in the artificial kidney before the experiment. **Conclusions:** On-line continuous blood glucose monitoring is feasible during hemodialysis and allows for a better metabolic state control of these patients. Hypoglycemia and hyperglycemia may be prevented by means of this method. The efforts involved in preparing this system before its use, as well as its costs can be reduced with the commercial production.

P4

Development of a percutaneous glucose monitor for continuous glucose monitoring

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A percutaneous device can be used as a carrier for a glucose sensor because of easy replacement and non-traumatic insertion of the sensor in case of impaired function. The purpose of this study was to investigate glucose kinetics in subcutaneous interstitial fluid collected by a percutaneous device.

The device consisted of a subcutaneous chamber in connection with a percutaneous part that penetrated the skin. A sintered titanium fibre mesh sheet was used for subcutaneous anchoring. The bottom of the device was covered with titanium fibre mesh or cellulose acetate to allow diffusion of tissue fluid. The devices were implanted in the backs of New Zealand white rabbits ($n=15$). Glucose kinetics were studied after injection of glucagon and octreotide. Four months after the start of the experiment, the animals were sacrificed for histologic evaluation of the implants and their surrounding tissue.

Basal glucose concentrations in tissue fluid varied widely. Injection of glucagon and octreotide caused a significant and prolonged increase in glycaemia. This increase was not directly followed by an increase in the glucose concentration of the subcutaneous interstitial fluid, but a clear delay was seen. In several cases no response was observed even up to 6 hours after injection. Histology was performed to relate these findings to the morphology of the tissue surrounding the implant.

This study was aimed at the direct collection of tissue fluid for validating measurements with an implantable glucose sensor. In contrast to previous experiments with implantable glucose sensors, no dynamic relationship was observed between glycaemia and glucose concentration in subcutaneous tissue fluid. Contributing factors in tissue reaction, implant design and glucose kinetics will be discussed.

P5

CRYOPRESERVATION OF PORCINE ISLETS IN UW SOLUTION CONTAINING POLYETHYLENEGLYCOL

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The **aim of the work** was to compare two cryopreservation agents for porcine islet conservation: DMSO and polyethyleneglycol (PEG). **Methods:** Porcine islets were isolated using a continuous collagenase digestion method and cultured for one week in Ham's F10 medium supplemented with 2% Ultrosor. They were subsequently cryopreserved either after being equilibrated with DMSO according to standard procedures or being suspended in UW solution containing 5% PEG 20kDa. After one night storage in liquid nitrogen, the islets were thawed and cultured over three weeks in Ham's F10 + Ultrosor. Insulin secretion in response to glucose was assessed in transwell microfilters, comparing insulin secretion over 45 minutes in Krebs buffer containing 50 or 180mg/100ml glucose. **Results:** 1) Under both cryopreservation conditions, insulin secretion index by thawed islets was ≥ 2 at all tested culture periods. 2) One week after thawing, stimulation index of PEG cryopreserved islets was 2.32 ± 0.64 ($p=0.041$ vs. 1, $n=7$), and was 2.25 ± 0.74 for non frozen control islets (NS, $n=7$). 3) At day 10, basal and stimulated insulin secretions were 90.9 ± 21.8 and $228.6 \pm 87.5 \mu\text{U}/45 \text{ min}$ for DMSO cryopreserved islets ($p=0.044$, $n=7$), and 243.4 ± 51.9 and $490.8 \pm 91.5 \mu\text{U}/45 \text{ min}$ for PEG cryopreserved islets ($p=0.026$, $n=7$). Insulin secretion was significantly higher for PEG-cryopreserved islets (vs. DMSO) both under basal ($p<0.01$) and stimulated condition ($p<0.001$). 4) PEG cryopreserved islets demonstrated a glucose concentration dependent insulin secretion after one and two weeks of culture (glucose tested concentrations = 0, 0.5, 1, 2 and 3g/l). **Conclusion:** 1) PEG-freezing-thawing procedure is faster and easier than the DMSO method. 2) Long term culture of thawed tissue yields functional islets. It can therefore be considered as a useful technique for porcine islet cryopreservation.